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TOXIC METALS IN *NEREIS DIVERSICOLOR* MÜLLER, 1776 FROM INNER SHORES IN SINOP PENINSULA OF THE BLACK SEA AS BIO-INDICATOR SPECIES

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ABSTRACT: The concentrations of Cd, Hg and Pb in the whole parts of the worm *Nereis diversicolor* collected from the Turkish Black Sea coast have been measured by ICP-MS (Inductively Coupled Plasma -Mass Spectrometer) for monitoring metal pollution in 2015. Seasonal differences in the amounts of Cd, Hg and Pb in the sediment were not significant. The amounts of Pb were found to be higher in sediment than those of *N. diversicolor*. On the other hand Cd and Hg amounts in the worms were higher than the sediment. However, there were significant differences in toxic metal levels between sizes of *N. diversicolor.* Larger specimen accumulated less toxic metal. Cd had the highest CF mean followed by Hg and Pb showed the lowest CF value. It is concluded that the worm *N. diversicolor* are suitable biomonitors to assess changes in metal pollution in this coastal area of the Black Sea.

KEYWORDS: *Nereis diversicolor*, bio-indicator, toxic metals, Black Sea.

INTRODUCTION

Pollution problem has become serious issue in the Black Sea due to the continuous pursuit of human population towards urbanization and industrialization. The Black Sea ecosystems are exposed to excessive input of pollutants from various sources such industrial and agricultural processes, domestic and sewage wastes, fishing and touristic activities and so on (Bat *et al.,* 2018a).

The uncontrolled discharge of pollutants into the coastal water directly affects aquatic organisms, including macro-benthic organisms, which are considered a bio-indicator of marine environmental pollution (Bat, 2005) since are known to accumulate toxicants (Bat and Arici, 2018). The significance of the use of bio-indicators to detect contaminants put forwarded by the Marine Strategy Framework Directive (Official Journal of the European Union, 2008). Macro-benthic organisms are used as bio-indicators, because these species are more stable than planktonic and pelagic organisms and they respond relatively rapidly to anthropogenic stress and contaminants especially heavy metals. Although the coastal area in the Black Sea is free of hydrogen sulphide, concentrations increase rapidly under the thermocline due to the restricted ventilation of deeper shelf water. Consequently, the number of macro-benthic species decreases rapidly with increasing depth. The study area (Sinop Peninsula) is located at the central part of the southern Black Sea. It is one of the most important natural harbours of the Black Sea and is characterized by high hydrodynamic conditions. Environmental parameters can significantly influence the diversity, density and structure of ecosystems. Moreover, human settlement along the coasts is very obvious that could cause serious threats to the marine life.

Marine pollution studies are essential to protect the coastal ecosystems. The determination of contaminants especially toxic metals in biota may help to understand current status of pollution. Studies on heavy metal accumulation in polychaetes of the Black Sea coasts are scarce (Bat *et al*., 2016) despite the sea has a rich fauna of Polychaeta.

On the other hand, Polychaeta, is a wide spread organisms in the Black Sea (Kurt-Sahin and Çinar, 2012; Çinar *et al*., 2014) that has a significant ecologic value in marine ecosystems. *Nereis diversicolor* Müller, 1776 is one of the commonest intertidal polychaetes. It lives in a rather permanent, often U-shaped, burrow in soft sediment (Fish and Fish, 1996). It is widely distributed in the Black Sea (Kurt-Sahin and Çinar, 2012; Çinar *et al*., 2014). *N. Diversicolor* is an opportunistic predator herbivore scavenger and hunter deposit-feeder suspension-feeder browser that spends its larval life in the surface covers of the sediments, and when larger builds big, branched burrow systems with several holes to the surface (Barnes, 1994). Also, the phytoplankton is confined on a mucous funnel secreted by *N. diversicolor* at the way to the burrow and ingested (Fish and Fish, 1996). The main predators of the worm are fishes and birds.

The main objectives of the current work were (1) to determine if accumulation levels of toxic metals in the whole parts of the worm and (2) to evaluate seasonal and sizes differences of these metals.

MATERIALS AND METHOD

Sampling site and Physico-chemical parameters of water:

Polychaetes were collected in Sinop shores of the Black Sea, Turkey (Fig. 1). Water analysis was done prior to the collection of *N. diversicolor* samples using multi-parameter tester (HORIBA model). Temperature, pH, TSS, salinity and dissolved oxygen of the water were determined. This was done three replicates in sites where polychaetes were collected.

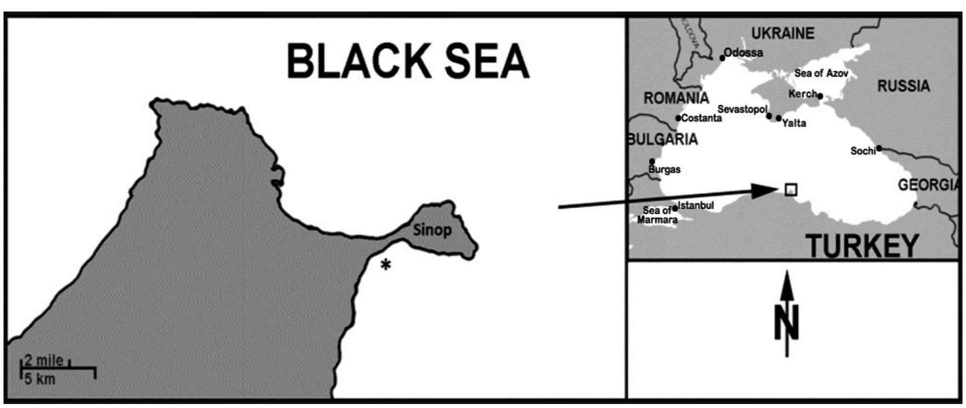

Fig.1. Study area.

Collection and analysis of sediment:

Sediment samples were collected in PVC cores by divers, in the vicinity of the worm *N. diversicolor.* The core samples obtained was 10 centimetres in length with a diameter of 4 cm. After the sediment collection, all of them were sliced 4 cm interval. The slices were put in cleaned polyethylene bags with on ice to keep cold and transferred to the Hydrobiology Laboratory of Fisheries Faculty, Sinop.

Two kg of sediment samples were taken from study area by quadrature method. Samples were placed in plastic bags. The samples were stored at -21°C. Organic carbon, particle size analysis, water content, porosity, pH and redox values of sediment samples were determined.

Sediment samples for total organic carbon analysis were dried at 105°C for 24 h. Five grams of dried sample were then treated with hydrochloric acid vapor overnight in a desiccating jar to convert any calcium carbonates to chlorides. Samples were then placed in a muffle furnace at 600°C for 4 hours. The loss ignition was taken as the organic carbon content of the sediment (Buchanan, 1984).

In order to determine the water content, dry weight was weighed by drying for 2 days. After cooling, water amount % calculated from weight differences.

For the purpose of determining the porosity value of the sediment samples, the samples were dried in 105°C for 48 h and then put in pure water for 24 h. Finally these samples were weighed again and the porosity values were calculated from weight differences.

For the pH analysis, 1: 2.5 percent pure water were added to the sediment samples taken in a certain amount and the samples were thoroughly mixed in the water. After 30 min sediments were expected to settle in water, pH was measured by pH meter.

Oxidation-reduction (Eh) values were measured with Portable redox-meter.

Collection of polychaetes samples:

Individuals of *N. diversicolor* were collected seasonally in 2015 in soft sediment from inner shores in Sinop Peninsula of the Black Sea, and then samples were transferred to the laboratory for further analysis. After collection, the worms were washed with clean seawater to remove any debris on them and stored in tanks with aerated clean seawater for 48 hours to empty gut contents. The worms are then divided into three groups as A (25-40), B (41-56) and C (57-72) mm in lengths and A (127-210), B (211-320) and C (321-510) mg in weights, respectively. Each group was kept separately in cleaned polyethylene bags until metal analyses.

Analysis for heavy metals:

N. diversicolor analysis: Metal analysis of the worm samples were performed using m-AOAC 999.10- ICP/MS method by accredited Environment Industrial Analysis Laboratory Services Trade Company (TURKAK Test TS EN ISO IEC 17025 AB-0364-T). EN 15763 European Standard methods were applied.

Sediment analysis: Cd and Pb in all subsampling pulverized to 85% passing 200 mesh was performed using 4 acid digestion and ultra-trace ICP-MS method by accredited ACME Analytical Laboratories Ltd. (Vancouver, Canada). For Hg analysis Ultra-trace Aqua Regia digestion method was used by accredited ACME Analytical Laboratories.

Statistical Analysis of Data:

Statistical analyses of data were carried out using Statistica version 7.0 software. A one-way analysis of variance (ANOVA) was applied, come after Duncan comparisons for

the source of statistically significant differences of metal concentrations by seasons and sizes for each of the metal investigated in the current work. The significance was set at 0.05 and P-values less of 0.05 were considered statistically significant (Zar, 1984). All values being expressed on mg kg^{-1} dry wt. basis.
The Concentration factors (CFs) were performed to know the efficiency of *N. diversicolor* to accumulate heavy metal from sediment and were calculated as overall mean heavy metal levels in the worms / overall mean heavy metal levels in sediment.

RESULTS AND DISCUSSION

Water quality is very important for the survival of benthic organisms.

Results of water quality in Sinop shores of the Black Sea are shown in Table 1. All parameters tested is in accordance with the standards set by Turkish Official Gazette (Official Gazette of Republic of Turkey, 1983) and regulations (Turkish Environmental Regulations Water Pollution Control Regulation, 2004–2005), as a results, the said Sinop shores is under favorable condition for the survival of polychaetes.

Table 1. Water quality of Sinop shores of the Black Sea.

Parameters	Spring	Summer	Autumn	Winter
Dissolved oxygen (mg/L)	4.93±0.1	4.85±0.1	5.22±0.1	5.44±0.1
Temperature (°C)	21.1±0.1	23.4±0.2	14.3±0.2	8.19±0.1
pH	8.83±0.1	8.72±0.1	8.85±0.1	8.81±0.1
Conductivity (uS/cm)	28391±14	28925±13	28435±11	28738±12
Salinity (‰)	17.4±0.3	17.5±0.3	17.3±0.2	17.6±0.2
TDS (g/L)	18.29±0.1	18.72±0.1	18.53±0.1	18.61±0.1

Data on organic carbon, water content, porosity, pH and redox values of sediment are given in Table 2.

Table 2. Organic carbon, water content, porosity, pH and redox values of sediment.

Parameters	Spring	Summer	Autumn	Winter
Organic carbon (%)	0.35±0.08	0.91±0.04	1.79±0.12	0.28±0.07
Water content (%)	31.4±0.2	23.1±0.1	37.2±0.2	43.1±0.2
Porosity	very	medium	very	medium
pH	7.55±0.1	7.93±0.1	8.61±0.1	7.14±0.1
Redox (Eh, Mv)	-121	-193	-220	-88

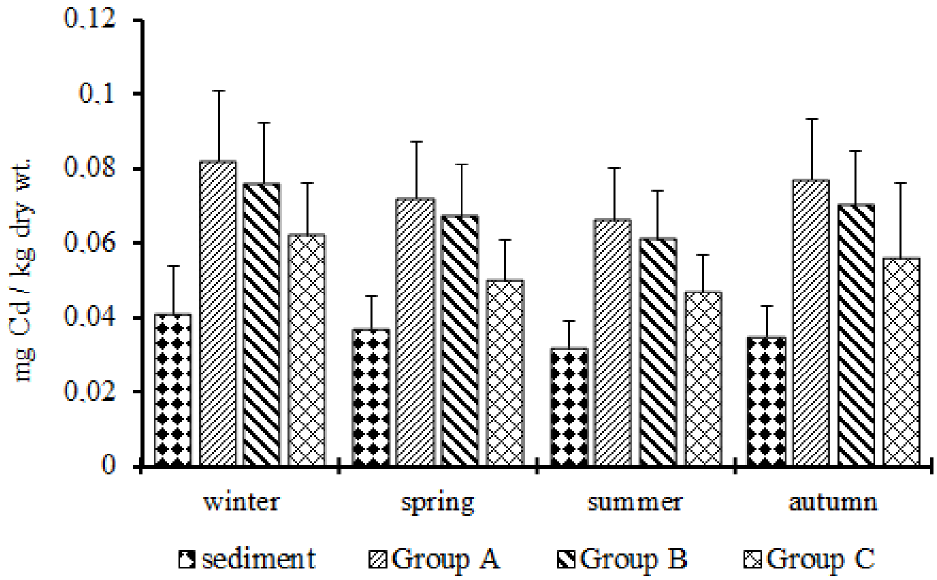

Fig. 2. The means with standard deviations (vertical line) of Cd amounts in the *N. diversicolor* and in sediment from Sinop inner shores of the Black Sea seasonally in 2015.

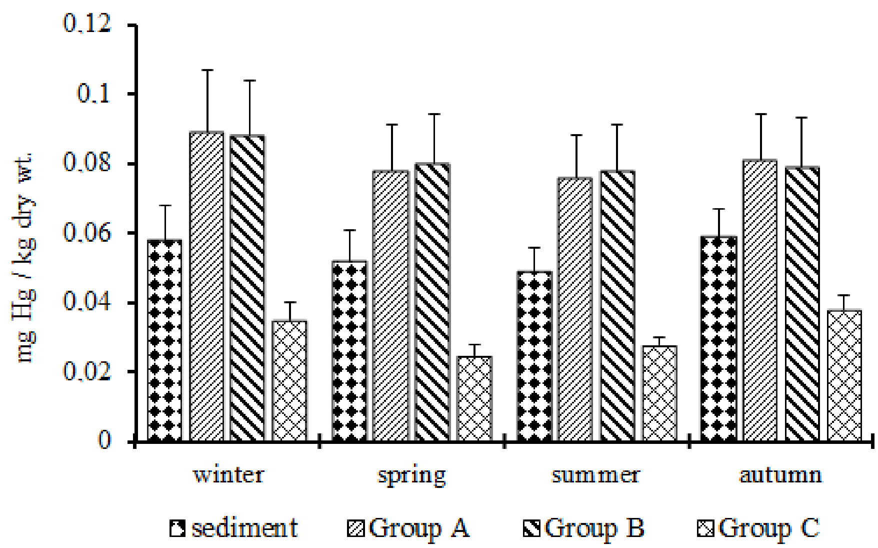

Fig. 3. The means with standard deviations (vertical line) of Hg amounts in the *N. diversicolor* and in sediment from Sinop inner shores of the Black Sea seasonally in 2015.

The levels of the heavy metals in the whole soft tissues of *N. diversicolor* and in sediment from Sinop inner shores of the Black Sea given in Figs. 2-4.

Although there are slightly seasonal differences in the amounts of Cd, Hg and Pb in the sediment, they are statistically insignificant ($p>0.05$). The amounts of Pb were found to be higher in sediment than those of *N. diversicolor*. On the other hand Cd and Hg amounts in the worms were higher than the sediment.

There was also no significant differences ($p>0.05$) in the metal levels in same groups of the worms between seasons, whereas different groups showed seasonally differences ($p<0.05$).

In general, the toxic metals showed a tendency to accumulate in *N. diversicolor*. There were significant differences in toxic metal levels between sizes of *N. diversicolor*. Larger specimen (Group C) accumulated less toxic metal. Groups A and B were accumulated significantly higher all the toxic metals than those in Group C. However it was found that Cd amounts in Group A were higher in whole tissues of *N. diversicolor* than those in Group B and Group C. But Hg and Pb amounts in both Group A and Group B showed no significant differences ($p>0.05$).

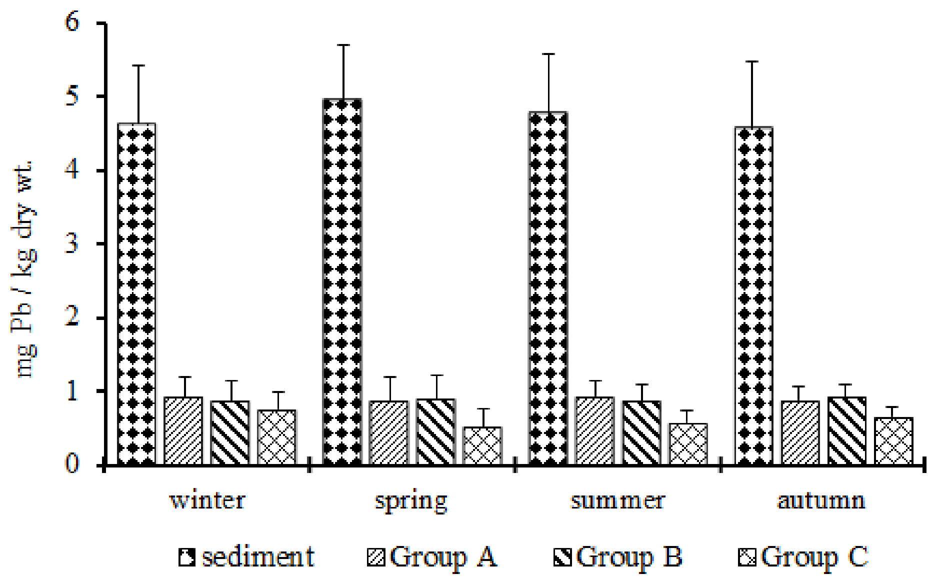

Fig. 4. The means with standard deviations (vertical line) of Pb amounts in the *N. diversicolor* and in sediment from Sinop inner shores of the Black Sea seasonally in 2015.

Differences in sizes may show differences in the amounts of the toxic metals in body tissues (Bat *et al*., 2018b). Differences in accumulation between the sizes may be attributed to differences in metal bioavailability, physico-chemical parameters the environment, changes in tissue composition, reproductive cycle, habitat, feeding habits, capacity of metals accumulation (Phillip and Rainbow, 1994). Breeding of

N. diversicolor has been found in spring at the times of new and fullmoon. Lifetime of the worms is probably about three years. They breed once then die (Fish and Fish, 1996). It was emphasized that that smaller and lighter specimen were found to contain significantly higher toxic metals than heavier specimen in the marine environment (Phillips, 1976). It was also pointed out that if growth was fast compared with metal accumulation, the observed metal levels would decrease with size, even though the overall metal content increased. This trend indicates those smaller specimens are more sensitive to heavy metal amount and hence have a greater tendency to accumulate than heavier species. On the other hand as the worm grow in size, the body amounts of heavy metals become more stable and get decreased. Bat *et al*. (2001) showed that the worm had a greater resistance to the contaminated water with clean sediment. It was indicated that this worm was a very robust organism, capable of tolerating quite high levels of Zn and Pb. They also found that small worms are more sensitive to Zn and Pb than bigger worms (Bat *et al*., 2001). Bryan and Hummerstone (1973) found that cadmium was not very toxic to *N. diversicolor*. Bryan (1974) also found that tolerant individuals of *N. diversicolor* were about four times more resistant than non-tolerant individuals.

The concentration factors (CFs) were made to evaluate the efficiency of three sizes groups of *N. diversicolor* to accumulate toxic metals from sediment. Low concentration factors of heavy metals are indicative of low accumulation of heavy metals by the worms whereas high concentration factors of the heavy metals indicate active uptake. Cd had the highest CF mean followed by Hg and Pb showed the lowest CF value (Fig. 5).

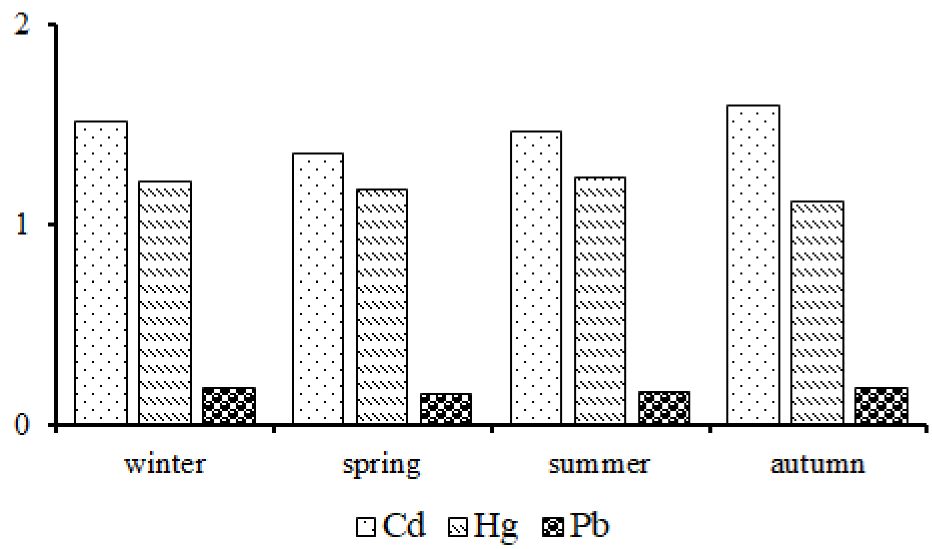

Fig. 5. Mean bio-sediment concentration factors (CF) in *N. diversicolor*.

It is also known that lesser than 63µm particles of sediment are the greatest significant sources of available metals (Bryan and Langston, 1992; Langston and Spence, 1994). If *N. diversicolor* ingests particles larger than > 63µm or avoids toxic metal contaminated sediments, this might account for the lower metal amounts observed in the whole tissues. Bryan and Hummerstone (1971) found the amounts of Cu, Zn, Pb, Mn and Fe in *N. diversicolor* and in sediments from seven English estuaries influenced to varying degrees by a copper-tin mineral zone. It was found that this worm appeared to regulate its Zn levels, but its Cu levels increased with increasing Cu levels in sediment. Bryan (1974) determined that the Cu, Pb and Cd amounts in *N. diversicolor* correlated strongly with amounts in sediments from several English estuaries. According to these results it can be concluded that benthic organisms are able to remove metals from the sediments (Bat and Raffaelli, 1998). However, when compared to abiotic processes, the accumulation of contaminants would seem to be a minor transport process (Swartz and Lee, 1980). Renfro (1973) reported that *N. diversicolor* at a density of 50 worms per m^2 would accumulate only 0.08% of the ^{65}Zn of the upper 2 cm of the sediment layer, whereas 3% of the ^{65}Zn would be lost from the sediment by resuspension.

CONCLUSION

This work was carried out to provide information on heavy metal amounts in sediment and in three sizes groups of *N. diversicolor* from inner shores in Sinop peninsula of the Black Sea as bio-indicator species. According to our results, we found that Cd, Hg and Pb levels were higher in smaller individuals (Groups A and B) in whole tissues of *N. diversicolor* than the bigger (Group C). In spite of that toxic heavy metal amounts in sediment and in *N. diversicolor* are low; a possible hazard may appear in the future depending on the domestic wastes and agricultural activities in the region. It is concluded that the area of this work is in general not considered toxic metal pollution. However, it is suggested that a continued monitoring programme for contaminant compounds in sediments and biota along the Black Sea coasts (Official Journal of the European Union, 2008). Such works will contribute the improving of an effective coastal management programme to keep safe the ecological integrity of this unique ecosystem and the health of humans associated with it.

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